Does depth become a permissive factor for reproductive habitat selection in the reef-associate damselfish *Chrysiptera cyanea*?

by

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Abstract. – The sapphire devil *Chrysiptera cyanea* (Quoy & Gaimard, 1825) is a reef-associated damselfish. Because this species exhibits repeated active reproduction in shallow seas during the spawning season, it is hypothesized that there is a preferred depth for successful reproduction. The aim of this study was to clarify whether the reproductive activity of the sapphire devil is influenced by habitat depth. In a field survey, many individuals could be seen in shallow areas (0-1.5 m), but few in deeper areas (> 3.1 m). Based on this observation, mesh cages containing one male and eight females were set at the surface (0 m; surface group) and the bottom (3 m; bottom group) of coral reefs during the spawning season and ovarian development in the two groups was compared. At the start of this experiment, all fish had a high gonadosomatic index (GSI) (6.33 ± 0.55) and ovaries with many vitellogenic oocytes at the tertiary yolk stage. Fish in the surface group maintained a high GSI (4.46 ± 0.83; 6.13 ± 0.91) and well-developed oocytes laden with yolk during the experimental period. Conversely, the GSI of the bottom group statistically decreased (1.73 ± 0.67; p = 0.0018) beginning three weeks after initiation of the experiment. In the bottom group, the number of vitellogenic oocytes in ovaries decreased gradually and, consequently, ovaries were occupied by immature oocytes at the perinucleolus stage. These results suggest that depth has an impact on ovarian development in the sapphire devil. Measurement of levels of dopamine (DA), its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), and DA metabolic rate (DOPAC/DA) in the brain using high-performance liquid chromatography (HPLC) together with an electrochemical detection system indicated that DA levels increased (p = 0.046) and the metabolic rate of DA (DOPAC/DA) decreased (p = 0.0036) in the bottom group. These findings suggest that the combination of light wavelength and hydrostatic pressure impacts the regulation of reproduction in fish inhabiting shallow waters.

Résumé. – La profondeur de l’eau est-elle un facteur permissif pour la sélection de l’habitat de reproduction chez un poisson corallien, la demoiselle bleue, *Chrysiptera cyanea* ?

La demoiselle bleue ou diable bleu, *Chrysiptera cyanea* (Quoy & Gaimard, 1825), est un poisson qui s’installe et vit dans les récifs coralliens. Cette espèce présentant une reproduction active répétée dans les eaux peu profondes pendant la saison du frai, on suppose qu’il existe une profondeur adéquate pour optimiser la reproduction. Le but de cette étude est de préciser si l’activité reproductrice du diable bleu est influencée par la profondeur de l’habitat. Lors d’une étude sur le terrain, plusieurs individus ont été aperçus dans les zones peu profondes (de 0 à 1,5 m), mais très peu dans les zones plus profondes (> 3,1 m). Partant de cette observation, des cages en filet contenant un mâle et huit femelles ont été placées à la surface (0 m ; groupe Surface) et au fond (3 m ; groupe Fond) des récifs coralliens pendant la saison du frai et le développement ovarien des poissons a été analysé afin de comparer les deux groupes. Au début de cette étude, tous les poissons avaient un indice gonadosomatique (GSI) (6,33 ± 0,55) élevé et les ovaires contenaient beaucoup d’ovocytes au stade du follicule tertiaire. Les poissons du groupe Surface ont maintenu un GSI (4,46 ± 0,83 ; 6,13 ± 0,91) élevé et présentaient des ovocytes bien développés riches en vitellus durant la période d’essai de trois semaines (1,73 ± 0,67 ; p = 0,0018). À l’inverse, le GSI des individus du groupe Fond a diminué à compter du 12^e jour après le début de l’expérience. Dans le groupe Fond, le nombre d’ovocytes présents dans les ovaires a diminué progressivement jusqu’à ce que les ovaires soient remplis d’ovocytes immatures au stade périnucléaire. Ces résultats suggèrent que la profondeur de l’habitat a un impact sur le développement ovarien chez le diable bleu. La mesure des niveaux de dopamine (DA), son métabolite 3,4-acide dihydroxyphénylacétique (DOPAC), et du taux métabolique de la dopamine (DOPAC/DA) dans le cerveau par chromatographie liquide à haute performance (HPLC) associé à un système de détection électrochimique a indiqué que les taux de DA (p = 0,046) et de DOPAC/DA (p = 0,0036) ont augmenté pour les individus du groupe Fond. Ces résultats suggèrent que la combinaison de la longueur d’onde de la lumière et de la pression hydrostatique a un effet sur la régulation de la reproduction chez les poissons habitant les eaux peu profondes.

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Fish inhabiting shallow waters are constitutively exposed to periodic changes in environmental parameters, which they utilize to synchronize reproductive events. Among a number of factors in aquatic environments, photoperiod acts as the proximate cue to initiate and terminate reproduction in the majority of temperate fish because it is the most reliable measure of calendar time at high latitudes (Bromage *et al.*, 2001). Using information obtained from long- or short-day conditions, fish repeat gonadal development and spawning at a fixed time of year through activation of the endocrine system in the hypothalamic-pituitary-gonadal axis.

Compound and depth-dependent factors, wavelength of light and hydrostatic pressure, influence the utilization of day length in shallow waters. Light of long wavelengths is readily absorbed by the water column and "the blue-rich world" spreads out in deeper waters. Therefore, it is possible that fish have a preferred spectrum of light to which they have adapted due to its presence in their natural habitat (Lythgoe, 1980). In this regard, experiments on melatonin synthesis using cultured pineal glands revealed that Atlantic salmon *Salmo salar* Linnaeus, 1758 is sensitive to green (550 nm) and blue (450 nm) light, but insensitive to red light (650 nm) (Vera *et al.*, 2010). Moreover, light of a wide range of wavelengths can be perceived by the Atlantic cod *Gadus morhua* Linnaeus, 1758 (Vera *et al.*, 2010), which is a benthic species exhibiting vertical migration into the upper pelagic zones. Therefore, wavelengths of light are a limiting factor in habitat selection. The second factor, hydrostatic pressure, is related to the tidal cycle, which repeats at 12.4-h intervals, and plays an important role in synchronizing reproductive and behavioural activities in certain fish species (Gibson, 1984; Takemura *et al.*, 2008, 2010a). It was reported that hydrostatic pressure has an impact on dopaminergic activity in the brain of certain fish (Damasceno-Oliveira *et al.*, 2006, 2007; Takemura *et al.*, 2010b, 2012). Because dopamine (DA) has an inhibitory impact on the hypothalamic-pituitary-gonadal axis in certain fish species (Zohar *et al.*, 2010), hydrostatic pressure is also a limiting factor in habitat selection. Therefore, it is hypothesized that fish select a habitat with a preferred wavelength of light and hydrostatic pressure to maximize their reproductive success.

The sapphire devil *Chrysiptera cyanea* is a reef-associated damselfish that is distributed widely in the shallow waters of the western Pacific Ocean. They form a group consisting of few males and several females in nature. Previous studies have revealed that long-wavelength-sensitive cone opsin is expressed in the brain (Takeuchi *et al.*, 2011) and that exposing fish to long-day conditions by means of red light-emitting diode (LED) light induces vitellogenesis (Bapary *et al.*, 2011; Takeuchi *et al.*, 2011). In addition, treatment of fish with a neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), resulted in acceleration of ovarian development (Badruzzaman *et al.*, 2015). Since MPTP is known to induce selective and irreversible destruction of the dopaminergic nigrostriatal pathway and lead to symptoms of parkinsonism in mammals (Burns *et al.*, 1983; Heikkila *et al.*, 1984), a decrease in DA content in the brain is likely related to ovarian development in the sapphire devil. Based on previous findings, the aim of the present study was to identify the physiological mechanism underlying the active reproduction of this species in shallow waters, which are rich in light of long wavelengths and have a low hydrostatic pressure. Gonadal development and dopaminergic activity in the brain were compared between matured fish groups reared at different depths. Fish were kept at the surface (0 m) and the bottom (3 m) of reef lagoon and gonadal activity was compared. Dopaminergic activity was also measured in the brains of fish reared under various hydrostatic pressure conditions.

MATERIALS AND METHODS

Field survey

The distribution of the sapphire devil in Odo (Itoman City), Miyagi (Chatan Town), Zanpa (Yomitan Village), and Bise (Motobu Town), Okinawa, Japan, was assessed from June to July 2013. At each location, corals with sapphire devil schools were identified around the time of mean sea level by snorkelling. When a school was confirmed, the number of adult fish was counted by eye and the depth of their habitat was measured using a dive computer (TUSA, Chiba, Japan). Depth was divided into the following three categories: 0 to 1.5 m (shallow), 1.6 to 3.0 m (middle), and more than 3.1 m (deep). Schools were scored by the number of individuals observed in a coral as follows: fewer than two individuals in a coral (0 points); three to five individuals in a coral (1 point); six to eight individuals in a coral (2 points); and more than nine individuals in a coral (3 points).

Effect of depth on gonadal development

Fish used in the present study were collected from a reef lagoon around Okinawa-Jima, Okinawa, Japan, using small nets in April of 2013 and transferred to outdoor tanks (500 L capacity) with running seawater and aeration under natural photoperiod and temperature conditions at Sesoko Station, Tropical Biosphere Research Center (TBRC), University of the Ryukyus, Okinawa, Japan. They were fed daily with a commercial feed (Fry Feed Kyowa C1000; Scientific Feed Laboratory, Tokyo, Japan). Their body mass ranged from 1.88 to 4.07 g.

In the present study, the effect of depth on gonadal development of the female sapphire devil was assessed. Considering the involvement of social cue from the male fish in the initiation of gonadal development in the female fish, one male and eight females in a cage were housed in 12 black mesh cages (25 × 25 × 25 cm) with a polyvinyl chloride pipe as a nest and acclimated at the surface of the lagoon (0 m; surface group) in front of Sesoko Station. After acclimation for several days, eight cages were set on the sea bottom (3 m; bottom group). The cages were removed every week after the start of the experiment. No food was provided during the experimental period. Fish (n = 8) reared in the outdoor tanks were used as controls.

Female fish in each cage were immediately anaesthetized in ice-cold seawater and their body mass was measured. An ovary and liver were removed from the body cavity and weighed. Pieces of ovary were fixed in Bouin's solution. The gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated using the following formula:

$$\mathrm{GSI} = (\text{ovarian mass}/\text{body mass}) \times 100$$

$$\mathrm{HSI} = (\text{liver mass}/\text{body mass}) \times 100$$

All experiments were conducted in compliance with the guidelines of the Animal Care and Use Committee of the University of the Ryukyus and the Regulations for the Care and Use of Laboratory Animals in Japan.

Effect of hydrostatic pressure on DA content in the brain

Four mesh cages (25 × 25 × 25 cm) were set at the water surface of a concrete tank of 3 m depth. To assess the effect of hydrostatic pressure on DA content in the brain in the absence of social cue from the male fish, eight females were housed in each cage and acclimated for several days. Two cages were kept at the water surface in the concrete tank, while the remaining two cages were moved to the bottom of the concrete tank. At 3 and 6 h after the start of the experiment, fish were removed from the cages and anaesthetised with ice-cold water. The body mass of fish was determined and the whole brain was collected and stored at –80°C.

Exposure to hydrostatic pressure under long-day conditions

Fish were collected in Bise (Motobu town), Okinawa, Japan, using small nets in November 2013 and transferred to outdoor tanks (500 L capacity) containing running seawater and aeration under natural photoperiod and temperature conditions at TBRC. Six cages were set at the surface of a concrete tank (100 metric ton capacity and 3 m in depth). To fulfil the social cue from the male fish, one male and six females were housed in each cage. After acclimatization, three cages were transferred to the bottom of the concrete tank. Long-day conditions (14 h light and 10 h dark) were set in each tank using a red LED light (OPTILED lighting International, Kwun Tong, Hong Kong). At 1, 2, and 3 weeks after transfer, fish were removed from the cages, anaesthetised in iced seawater, and weighed. Ovaries were also removed, weighed, and used for measurement of GSI and histological evaluation. The mean water temperature during the experimental period was 25.3°C.

Histological assessment

Pieces of ovary were dehydrated in a graded ethanol series, cleared with xylene, and embedded in histoparaffin (Paraplast plus; Sigma-Aldrich, St. Louis, MO). After sectioning at 7 µm and staining with Delafield's hematoxylin–eosin, ovarian histology was observed by microscopy. According to Bapary *et al*. (2009), sapphire devil oocyte development can be classified into the following stages: the peri-nucleolus stage (PNS), oil-droplet stage (ODS), primary yolk stage (PYS), secondary yolk stage (SYS), and tertiary yolk stage (TYS).

Monoamine measurement

According to the method of Takemura *et al*. (2010b), levels of DA and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), were measured using high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) with an SC-5ODS column (0.45 × 15 cm, EICOM, Kyoto, Japan). Reference standards for DA, DOPAC, and isoproterenol HCl (ISO; an internal standard) were purchased from Sigma-Aldrich.

Brain samples were homogenized in ice-cold 0.2 M perchloric acid (Wako Pure Chemical Industries, Osaka, Japan) containing 0.2 ng/mL ISO. The homogenate was kept on ice for 30 min and then centrifuged at 20,000 *g* for 15 min at 4°C. The supernatant was passed through a 0.45 mm filter (Minisart RC4, Sartorius AG, Goettingen, Germany). An aliquot (5 mL) of the filtrate was applied to the HPLC column, which had been equilibrated with 0.1 M acetic-citric buffer (pH 3.5) consisting of 17% (v/v) methanol, 190 mg/L sodium octylsulphate (Wako Pure Chemical Industries) and 5 mg/L ethylenediaminetetraacetic acid disodium salt dihydrate (Sigma-Aldrich). The isocratic flow rate was set at 0.5 mL/min. The profiles of DA, DOPAC, and ISO were evaluated using an electrochemical detector (ECD-300, EICOM), and analyzed using the LC solution software (Shimadzu). DA, DOPAC, and ISO levels in the brain were expressed in relation to the wet mass of brain tissue.

Statistical analyses

All data are expressed as means ± standard error of the mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) or two-way ANOVA followed by Tukey-Kramer comparisons of multiple groups. Statistical significance was accepted for values of $p < 0.05$ in the present study.

RESULTS

Distribution of the sapphire devil

The distribution of sapphire devil schools in coral reefs is shown in figure 1. A total of 97 schools was found. Accord-

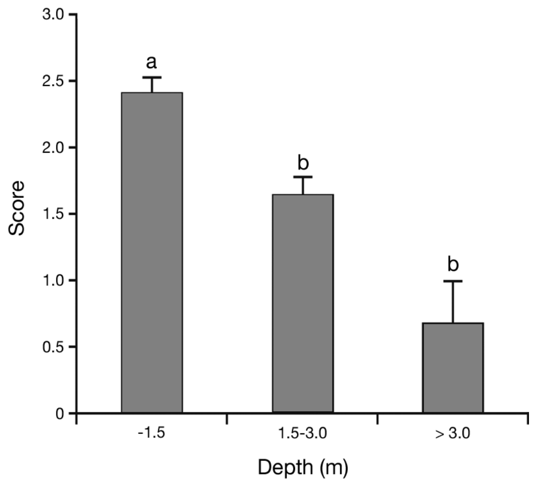

Figure 1 – Distribution of the sapphire devil in reef lagoons. School was scored according to the number of fish ($n \leq 2$; 0, $3 \leq n$ 5; 1, $6 \leq n \leq 8$; 2, $9 \leq n$; 3). Each value represents mean ± SEM. Different letters indicate significant difference among groups at $p < 0.05$.

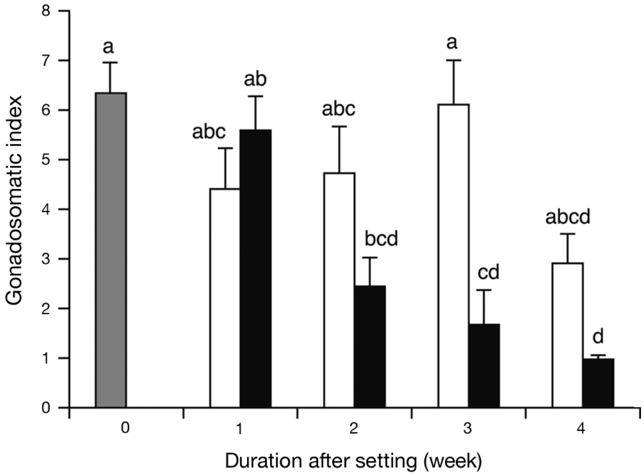

Figure 2 – Effect of rearing depth in reef lagoon on gonadosomatic index (GSI) of female sapphire devil. Mesh cages with fish (1 male and 8 females) were set at the surface (0 m; white column) and at the bottom (3 m; black column) of reef lagoon. Grey column indicates initial control. Fish in the cases were sampled every week after the onset of the experiment. Each value represents mean ± SEM. Different letters indicate significant difference among groups at $p < 0.05$.

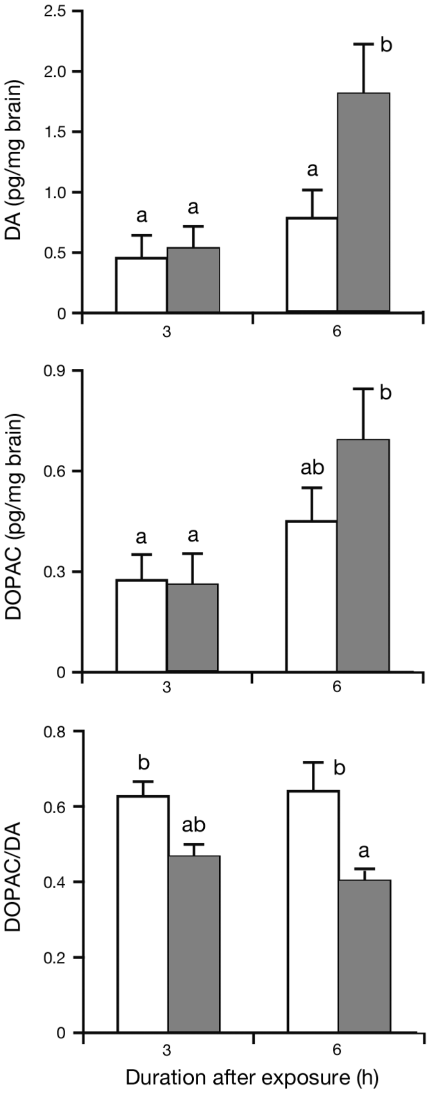

Figure 3 – Effect of hydrostatic pressure on dopaminergic activity in the whole brain of the sapphire devil. Mesh cages with 8 females were set at the surface (0 m; white column) and at the bottom (3 m; black column) of a concrete tank. Fish in the cages were sampled at 3 and 6 hours after the onset of the experiment. Dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the whole brain were measured high-performance liquid chromatography with an electrochemical detection system. Metabolic rate (DOPAC/DA) was also calculated. Each value represents mean ± SEM. Different letters indicate significant difference among groups at $p < 0.05$.

ing to habitat depth, 54, 40, and 3 schools were found at depths of < 1.5 m (mean score; 2.41), 1.6-3.0 m (1.65), and > 3.1 m (0.67), respectively. The score for the shallow school (< 1.5 m) was significantly higher than those of the intermediate (1.6-3.0 m) and deeper (> 3.1 m) schools.

Addition of hydrostatic pressure in field

Fish were reared in cages placed at the sea surface (0 m; surface group) and bottom (3 m; bottom group) and sampled every week (Fig. 2). On day 0 (initial control), the GSI of the initial control was high (6.33 ± 0.55) and vitellogenic oocytes at the TYS were observed within ovaries (Tab. I). The GSI of the surface group remained high from week 1 (4.46 ± 0.83) to 3 (6.13 ± 0.91). This value decreased at week 4 (2.95 ± 0.58), albeit not significantly. The gonadal histology of the surface group from week 1 to 3 was similar to that of the initial control. No vitellogenic oocytes were observed in ovaries of the surface group at week 4. Conversely, the GSI in the bottom group decreased throughout the experimental period. At week 3, the GSI of the bottom group was significantly lower than that of the surface group (1.73 ± 0.67, p = 0.0018). Vitellogenic oocytes at the TYS were observed in ovaries of the bottom group at week 1. However, ovaries of this group were occupied by immature oocytes at the PYS beginning on week 2 (Tab. I).

HSI was monitored throughout the experimental period. There was no significant difference in the HSI at weeks 3 and 4 (data not shown).

Effect of hydrostatic pressure

The effect of hydrostatic pressure for 3 and 6 h on dopaminergic activity in the brain was evaluated. There were no significant changes in DA and DOPAC levels, or DOPAC/DA between the surface and the bottom groups at 3 h. However, exposure of fish to high hydrostatic pressure resulted in a significant increase in DA (p = 0.046) and decrease in DOPAC/DA (p = 0.0036) (Fig. 3).

Table I. – Effect of hydrostatic pressure on ovarian conditions in the sapphire devil. Presence and absence of developing oocytes in an ovary was expressed as "+" and "-", respectively. [1] PNS: perinucleolus stage; ODS: oil-droplet stage; PYS: primary yolk stage; SYS: secondary yolk stage; TYS: tertiary yolk stage.

Individuals	Duration (weeks)	Oocyte stage[1]				
		PNS	ODS	PYS	SYS	TYS
Initial control	0	+	+	+	+	+
Surface	1	+	+	+	+	+
	2	+	+	+	+	+
	3	+	+	+	+	+
	4	+	–	–	–	–
Bottom	1	+	+	+	+	+
	2	+	–	–	–	–
	3	+	–	–	–	–
	4	+	–	–	–	–

Effects of long-day conditions with hydrostatic pressure

Fish in the surface and bottom groups were exposed to long-day conditions using red LED light plus hydrostatic pressure for three weeks. During the experimental period, the GSI of both groups remained low (0 m after 3 weeks: 0.57 ± 0.07; 3 m after 3weeks: 0.63 ± 0.05) (Fig. 4). Oocytes at the PNS were observed in all ovaries (data not shown).

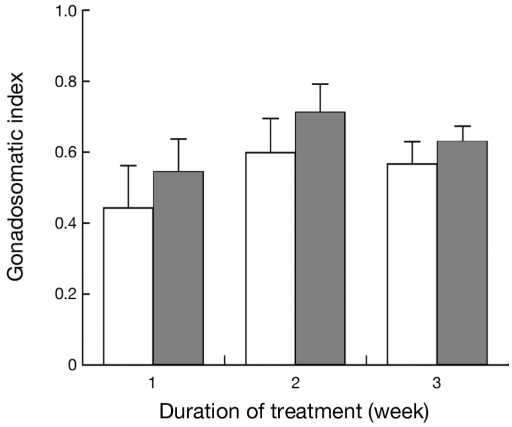

Figure 4 – Effect of long-day conditions with hydrostatic pressure on gonadosomatic index of the sapphire devil. Fish were reared under conditions with long-day (LD = 14 : 10) by red LED lights and with hydrostatic pressure at 0 m (white column) and 3 m (black column). Fish were sampled at 1, 2, and 3 weeks after the initiation of the experiment. Each value represents mean ± SEM.

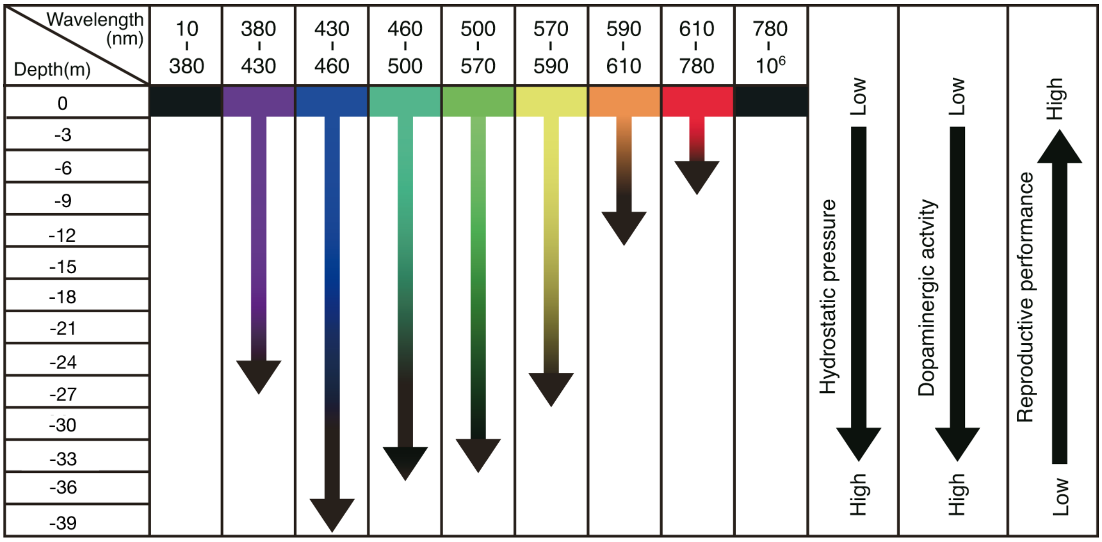

Figure 5. – Summary of depth effect on light wavelength, hydrostatic pressure, dopaminergic activity in the brain and reproductive performance of the sapphire devil. Long wavelengths of light are absorbed in the shallow area. Deeper habitat increases hydrostatic pressure, which follows an increase in dopaminergic activity in the brain and a decrease in reproductive performance in the sapphire devil.

DISCUSSION

Our field survey demonstrated that the distribution of sapphire devil communities is limited in shallow water during the breeding season. This suggests that the existence of optimal conditions for this species, although the observation that few structures where the sapphire devil schools are able to settle down were found in deep area might be one of the reason why the fish prefer to shallow water. Redding and Patiño (1993) stated that light wavelengths, water temperature, and hydrostatic pressure are proximal factors that can change according to depth. Water temperature and photoperiod during the experimental period ranged between 25 and 28°C and between 13 and 14 h, respectively. Since these two factors fluctuated within a suitable range for the initiation and continuation of reproductive activity in the sapphire devil (Bapary and Takemura, 2010), they likely had little impact on the results of this study. Light of long wavelengths can reach shallow water, where sapphire devil schools are abundant, and so may represent a proximal factor in their habitat. In this regard, *in situ* hybridization revealed that, in addition to the retina, mRNA of long-wavelength-sensitive cone opsin (*sdLWS*) is expressed in the third ventricle periventricular area in the anterior hypothalamus, including the ventromedial thalamic nucleus (VM), the anterior periventricular nucleus (NAPv), and the suprachiasmatic nucleus (NSC) (Takeuchi *et al.*, 2011), where light that penetrates to the deep brain is directly perceived (von Frisch, 1911). It was reported that sapphire devils during non-breeding season reared under long-day conditions using LEDs producing red light (627 nm), green light (530 nm), blue light (450 nm), and white light (5000 K), vitellogenic oocytes at the TYS were efficiently induced in ovaries by the red LED light (Bapary *et al.*, 2011). Therefore, it is possible that the sapphire devil perceives light of long wavelengths through the eyes and/or brain and utilizes it to synchronize reproduction because light of such wavelengths is abundant in their natural habitat. However, previous data do not rule out utilization of light of shorter wavelengths because other opsins, including vertebrate ancient *long* (VAL) opsin, rhodopsin, and exorhodopsin, were found to be expressed in the eyes and brain of the sapphire devil (Takeuchi, 2012). This may be supported by previous reports that green LED light is moderately effective in terms of inducing vitellogenic oocytes within ovaries (Bapary *et al.*, 2011, 2012; Imamura *et al.*, 2014).

Sapphire devils kept under hydrostatic pressure at 3 m depth exhibited increased DA and decreased DOPAC/DA levels in the brain. It is suggested that hydrostatic pressure alters monoamine levels and their metabolic rate in the brain of this fish. It has been reported that hydrostatic pressure influenced monoamine levels in the brains of the European eel *Anguilla anguilla* (Linnaeus, 1758) (Sébert *et al.*, 1986, 2008), the European flounder *Platicthys flesus* (Linnaeus, 1758) (Damasceno-Oliveira *et al.*, 2006, 2007), the threespot wrasse *Halicoeres trimaculatus* (Quoy & Gaimard, 1834) (Takemura *et al.*, 2010b, 2012), and the goldfish *Carassius auratus* (Linnaeus, 1758) (Ikegami *et al.*, 2015), although the effect of hydrostatic pressure on dopaminergic activity differs among species. For example, application of hydrostatic pressure at 1000 m depth caused increases in the contents of DA and epinephrine (E) in the brain of the European eel (Sébert *et al.*, 1986), which undergoes a spawning migration from their habitats to the Sargasso Sea. In contrast, cyclic changes in hydrostatic pressure at intervals of 12.4 h decreased the DA contents in the brain of the European flounder (Damasceno-Oliveira *et al.*, 2007), which undergoes repeated migrations between shallow and deep water according to the tidal cycle. In addition, application of hydrostatic pressure mimicking high tide resulted in a reduced level of DOPAC, a metabolite of DA, and a lower metabolic rate of DA (DOPAC/DA) in the brain of the threespot wrasse (Takemura *et al.*, 2010b, 2012), which spawns around high tide. These changes in dopaminergic activity in the brain of the threespot wrasse were associated with an increase in *in vitro* production of 17α,20β-dihydroxy-4-pregnen-3-one, but not of estradiol-17β, in cultured ovarian pieces in the presence of human chorionic gonadotropin (Takemura *et al.*, 2012). In this regard, DA is known to inhibit the release of gonadotropin from the pituitary and gonadotropin-releasing hormone (GnRH) from GnRH neurons in certain fish (Zohar *et al.*, 2010). It is possible that hydrostatic pressure drives dopaminergic activity, which in turn downregulates higher components of the hypothalamic-pituitary-gonadal axis of the threespot wrasse.

Recently, Badruzzaman *et al.* (2015) reported that sapphire devils treated with MPTP exhibit accelerated growth of vitellogenic oocytes within ovaries. Since MPTP destroys dopaminergic neurons (Goping *et al.*, 1995; Matsui *et al.*, 2009), the acceleration of vitellogenesis is likely due to a decrease in DA levels in the brain. Indeed, it was observed that MPTP treatment lowers dopaminergic activity in the whole brain as well as the level of tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis, in the diencephalon (Badruzzaman *et al.*, 2015). Therefore, since it is possible that hydrostatic pressure influences the release of GnRH and/or gonadotropin due to its effect on dopaminergic activity, the reproductive activity of the sapphire devil in shallow reef areas may be limited. This notion is supported in part by the results of MPTP treatment.

In conclusion, wavelength of light and hydrostatic pressure together are possible to play a role in the regulation of ovarian development in the sapphire devil (Fig. 5), and such physiological responsiveness might be involved in the fact that the sapphire devil prefers shallow reef areas. However, other unknown factors derived from the hydrostatic pressure treatment might be involved in gonadal development. Additionally, we failed to induce gonadal development under long-day conditions with a low hydrostatic pressure in the present study. This may be due in part to low water temperature because the experiment was carried out in November. Further studies are needed to clarify the factors important for control of reproductive events in the sapphire devil.

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